IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Gregory et. al.	Examiner:	Dr. K. C. Carlson
Serial No.:	08/087,132	Art Unit:	1812
Filed:	July 2, 1993	Docket:	NZI-012CN IG4-9.2(FWC)

For: NEW DIAGNOSTIC AND TREATMENT METHODS INVOLVING THE CYSTIC FIBROSIS TRANSMEMBRANE REGULATOR

Honorable Commissioner of
Patents and Trademarks
Washington, DC 20231

Declaration "F" of Dr. Richard J. Gregory under 37 CFR 1.131

SIR:

Dr. Richard J. Gregory declares as follows:

1. I am an inventor of the claims of the above-identified patent application and of the subject matter described therein.

2. I received my doctoral degree from the University of Massachusetts in 1986 and was a Principal Scientist at Genzyme Corporation in Framingham, MA during the time that the inventions described in the above-identified patent application were made.

3. I am a co-inventor of the present U.S. Application Serial No. 08/087,132 and of the inventions defined by the claims that are presently pending therein, and a co-inventor also of all of the parent applications thereof — No. 07/613,592, filed on November 15, 1990, No. 07/589,295 filed September 27, 1990, and No. 07/488,307 filed March 5, 1990.

4. This Declaration is being provided in order to make of record certain of our experiments that relate to the making of the T936C mutation in CFTR cDNA in order to stabilize the cDNA for propagation in E. coli, so that the cloned cDNA could then be expressed from a eucaryotic cell. These experiments were conducted in 1990 prior to the effective filing dates (or publication dates) of the following references:

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(A) M.L. Drumm et al., "Correction of the Cystic Fibrosis Defect in vitro by Retrovirus-Mediated Gene Transfer", Cell, 62, **September 21, 1990**, pp. 1227-1233.

(B) L. Tsui et al., "Stable Propagation of Modified Full Length Cystic Fibrosis Transmembrane Conductance Regulator Protein cDNA in Heterologous Systems", International Patent Application Number PCT/CA91/00341 published on April 2, 1992, bearing Publication Number WO 92/05252, and claiming the priority of Great Britain national application 9020632.7 **filed September 21, 1990.**

(C) United States Patent 5,240,846 to Collins et al. issued August 31, 1993 from Application No. 07/584,275 **filed September 18, 1990.**

5. The below-described experiments further demonstrate that we were in possession, prior to the above-mentioned dates in September 1990, of inventions defining CFTR-encoding DNA stabilized for propagation in E. coli , including stabilization achieved through the use of a point mutation that inactivates the cryptic bacterial RNA polymerase promoter positioned at nucleotides 908-936 of CFTR-encoding cDNA.

With respect to copies of laboratory notebook pages that are attached as exhibits to this Declaration, the page numbers and dates thereof have been removed as is permitted by PTO practice. The original notebook pages were properly witnessed in due course, and those dates also have been removed.

6. Exhibit 1 is a page from my laboratory notebooks (as maintained by the Genzyme Corporation) that was properly dated by me on a date that is prior to July 27, 1990, the date that our publication Richard J. Gregory et al., "Expression and Characterization of the Cystic Fibrosis Transmembrane Conductance Regulator", Nature , 347, issue of September 27, 1990, pp. 382-386, was received by that journal for publication. [The July 27, 1990 receipt date and the August 31, 1990 acceptance date of this article are clearly printed on the last page the publication — which is already of record herein via an Information Disclosure Statement, and additionally as an attachment to Declaration "D" submitted on June 9, 1995.]

7. Exhibit 1 clearly presents one of our inventions — that a T to C mutation at nucleotide position 936 in the CFTR-encoding cDNA will inactivate the cryptic promoter located at nucleotides 908-936 thereof, thereby stabilizing the DNA for propagation in E. coli.

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8. Exhibit 2 is a further page from my laboratory notebooks that was properly dated by me on a date that is prior to September 18, 1990, and shows our successful placement of a CFTR-encoding cDNA that contains the T936C mutation into a eukaryotic expression plasmid. The CFTR nucleotide sequence included therein extends from the codon for the CFTR initiator methionine (nucleotide 133) to well past the codon for the C-terminal amino acidthereof. Placement of the CFTR-encoding cDNA in the Vaccinia system expression plasmid was made possible by the T936C mutation, which had first permitted propagation (cloning) of a construct containing the full length cDNA in E. coli.

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9. All of the inventions disclosed in our present application and in all of the parent applications thereof were made in the United States.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of United States Code, and that the making of any such willful false statements may jeopardize the validity of this application, of related applications, and of any patent issued thereon.

Date: 8/15/95

Declarant: Richard J. Gregory
Richard J. Gregory, Ph.D.

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Exhibit 1

TLE

Project No.

Book No.

em Page No.

Mutagenesis of CFTR bacterial promoter.

An obvious way for me to stabilize the cryptic promoter now that we have localized it is by site directed mutagenesis to remove the promoter activity. In order to do this I had Gary synthesize the oligo below which will mutate T936 to a C.

enzyme DNA SYNTHESIS REQUEST FORM

Name: Rich Gregory Date:

Project: CFTR Project Cost # 62 Phone:

Oligomer name: CFTR-mutant T-936 Oligomer length: 20

Oligomer Use: (circle) probe (mutagenesis) primer-sequencing primer-pcr cloning

Sequence of Oligomer 5' - 3' (Note: use "N" for a mixed base and "I" for inosine) :

Example: GIANT or GIANT

CAGIATTIGGAITGTITTICABITC

DNA Chemistry Use Only:

Oligomer Name: 142kRG Date of Synthesis: By:

Column position : (1) 2 3 Last Trityl: On (Off)

column Id#: 22467

Purification: Extraction OPC Sep-Pak HPLC: 1 2 Gel: 12% 20%

OD260: 0.255 OD280: 0.148 (1/100dil) Concentration (mg/ml): 0.892

Date completed: By:

Note: DNA Chemistry uses an extinction coefficient of 1 OD = 35 ug/ml and retains 10% of the final product. Only 20% of the total is HPLC purified unless more is requested.

Notes:

VAX entered:

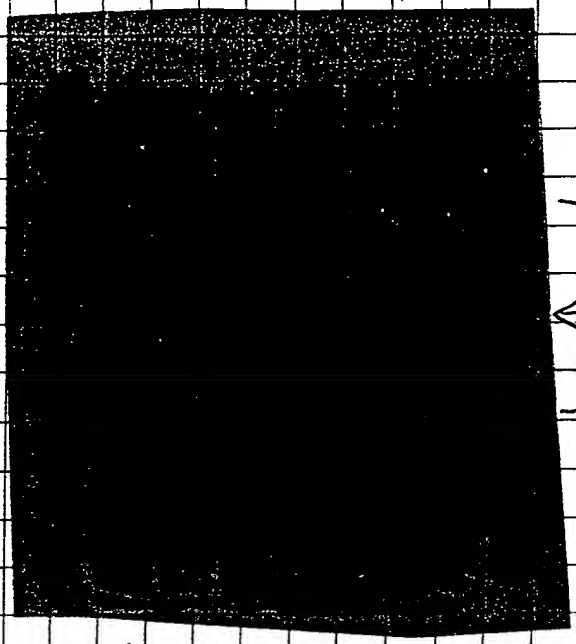
In the meantime I transformed some T16-1 into DH5αF'IQ and made single stranded plasmid DNA by diluting 5ml of O/N T16-1 DH5αF'IQ into 50 ml LB and adding 1 ml of R408 helper phage (5×10^{10} pfu/ml), growing 8 hours and performing standard single stranded DNA isolation final resuspension in 25 µl TE.

1 µg of oligo phosphorylated in 80 µl kinase rxn buffer for 1 hour at 37°C. Heat killed at 70°C for 20 min.

Mutagenesis performed using new USB T7-Gen kit with protocol on following page

To Page No.

tnessed & Understood by me,	Date	Invented by	Date

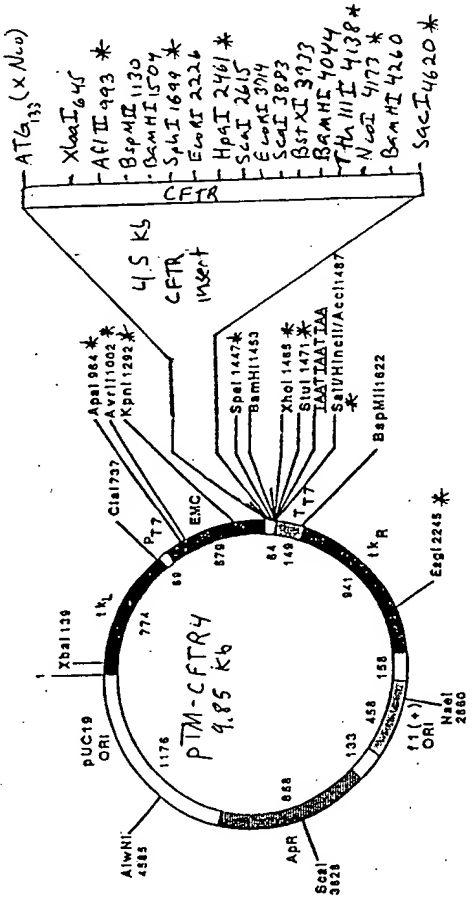


C1 = screwed up pTM-CFTR4
C2 = pTM-CFTR3
1-4½ clones

All 4 give CF of correct size so it looks like the problem is finished

PLASMID NAME: pTM-CFTR4
SIZE: 9.85 Kb
HOST CELL: AG1
DESCRIPTION: A high copy number CFTR expression plasmid for the vaccinia system containing the C490 mutation which allows high copy number and the CFTR sequences between the ATG at 133 and the SacI site at 4620. Mutation constructed in TM-1 the transferred as an XbaI/AflII fragment to pTM-CFTR3 pol3 AC and then into pTM1 as a ApaI/SacI fragment

LOCATION: DC-37/6Dk-42
DATE:
NOTEBOOK/PAGE: 1347/167-168, 179-182, 189-191
TECHNICIAN: R.B. Gregory
MAP:



* = unique site

No sites for:
Aat II NsiI
Asi HI SacI
Bst EII SfiI
MluI SnaBI
NarI SmaI
NdeI PstI
NheI XmaI
NotI

Witnessed & Understood by me, Date

Invented by Date

Recorded by

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Gregory et al.	Examiner:	Dr. K. C. Carlson
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For: NEW DIAGNOSTIC AND TREATMENT METHODS INVOLVING THE CYSTIC FIBROSIS TRANSMEMBRANE REGULATOR

Honorable Commissioner of
Patents and Trademarks
Washington, DC 20231

Declaration "E" of Dr. Alan E. Smith under 37 CFR 1.131

S I R:

I, Dr. Alan E. Smith, declare as follows:

1. In 1970 I received a doctoral degree in Molecular Biology from the Medical Research Council, Laboratory of Molecular Biology, Cambridge University. At all times that are relevant herein, I have been the Senior Vice President of Research of the Genzyme Corporation, the Assignee of record of the present patent application, and of all of the parent applications thereof. At Genzyme, I have been responsible for directing our gene therapy program including all research related to the provision of therapies for cystic fibrosis.

2. I have read and am familiar with our above-identified patent application, the parent applications thereof, and the content of the Official Action of December 9, 1994 that is pending herein.

3. This Declaration is being provided in order to facilitate the Examiner's anaylsis of certain oral remarks made in April and June of 1990 by Dr. Lap-Chee Tsui, a co-author /co-inventor of numerous of the references in the Exhibit Book submitted herein on June 9, 1995 including the reference J. Riordan et al., "Identification of the Cystic Fibrosis Gene: Cloning and Characterization of Complementary DNA", Science, 245, 1989, pp. 1066-1073, Document (B) in the Exhibit Book (hereinafter Riordan et al. 1989).

— Signature Copy —

4. The paragraphs of this Declaration are organized as follows:

(A) description of inventions (and the timeframe thereof) made prior to **July 27, 1990** by Genzyme inventors relating to stabilization of the CFTR-encoding cDNA against the effects of the cryptic bacterial RNA polymerase promoter that we determined to be present therein;

(B) discussion of the scientific mistakes in, and confusing nature of, the British patent application of Dr. Tsui et al. entitled "Stable Propagation of Modified Full Length Cystic Fibrosis Transmembrane Conductance Regulator Protein cDNA in Heterologous Systems" (which relates to the making of point mutations in CFTR-encoding cDNA), as filed on **September 21, 1990**; and

(C) discussion of certain oral remarks made by Dr. Lap-Chee Tsui at conferences held in **April and June of 1990** (preliminary to the September 21, 1990 British filing) that concern the making of point mutations in CFTR-encoding cDNA, but which were, for reasons described below, confusing and self-contradictory and therefore did not disclose any inventions to the art-skilled audiences present at those conferences.

Part (A) — Genzyme Inventions

5. Researchers in our company commenced assembly of a full length CFTR-encoding DNA sequence when it became apparent that, for unknown reasons, authors/ inventors having commonality with those of Riordan et al. 1989 were unable to announce construction of any full-length CFTR-encoding DNA.

6. Accordingly, following the publication date of Riordan et al. in 1989, Dr. Richard Gregory and his co-inventors at Genzyme obtained partial cDNA clones T11, T16-1, T16-4.5, and C1-1/5 from the American Type Culture Collection Rockville, MD, as deposited therein by Riordan et al. 1989.

7. As described more fully in Declaration "C" of Dr. Richard Gregory made of record herein on June 9, 1995, at a time prior to the March 5, 1990 filing date of our 07/488,307 parent application, Dr. Gregory had performed an analysis that identified two nucleotide subsequences in exon 6 of the CFTR-encoding cDNA (the first between about positions 748 and 778, and the second between about positions 908 and 936) that have strong sequence homology with the consensus nucleotide sequence for an E. coli RNA polymerase promoter.

The identification of those probable promoters (and of the apparent ribosome binding sites, i.e. Shine-Dalgarno sequences, and potential translation initiator methionines, all of which are appropriately positioned downstream therefrom) is clearly presented in Dr. Gregory's laboratory notebook page which bears a date prior to that of our **March 5, 1990** filing, and which was attached as an Appendix to the above-mentioned Declaration "C".

8. Examples 1 and 2 of the present Specification (see also Examples 2 and 3 of our parent 07/488,307 Specification filed **March 5, 1990**) describe experiments that were conducted in order to provide CFTR-encoding cDNA in a form that was stable to propagation in E. coli. As described in those Examples, placing the cDNA in a plasmid that was maintained in E. coli at very low copy number permitted stable propagation. These experiments are also believed to represent the first successful assembly of a single DNA that encodes for CFTR. The success of these experiments was consistent with the presence of such a promoter within the CFTR-encoding cDNA, and further explained the failure of all others, including the authors of Riordan et al. 1989, to announce isolation or construction of a full length encoding cDNA.

9. Prior to the filing of our 07/488,307 U.S. patent application on March 5, 1990, Dr. Gregory and his co-inventors at Genzyme provided a further demonstration that the CFTR cDNA could be stabilized —against the toxic effects of the cryptic promoter—for propagation in E. coli. Dr. Gregory and his co-inventors were able to disrupt production of toxic CFTR-derived polypeptides from the CFTR cDNA by placing therein an intervening sequence (an intron) downstream from, but still reasonably close to, the region of exon 6 that is referred to in paragraph (7) above . The intron was placed at the natural exon/intron boundary represented by nucleotide positions 1716/1717, which also provided a convenient cloning site. As confirmed in those experiments (which are more fully discussed in Dr. Gregory's Declarations "B" and "C" made of record herein on June 9, 1995), growth characteristics were improved (see Example 4, page 15, lines 7-11 of the 07/488,307 parent application filed March 5, 1990, and also Example 3, page 15, lines 1-3 of the present application) for host E. coli cells containing the new stabilized cDNA.

10. Accordingly, prior to the **March 5, 1990** filing date of our parent U.S. patent application, Dr. Gregory and his co-inventors had recognized—ahead of all others—that CFTR-encoding DNA contained a bacterial RNA polymerase promoter sequence, and that this was responsible for both the failure of Riordan et al. 1989 to have obtained a full-length CFTR cDNA during their original cloning experiments (see also Document A in the Exhibit Book), and during their subsequent efforts to isolate same.

11. Further confirmation of this fundamental discovery was provided by the straightforward and merely routine experiments described in the publication of Dr. Gregory et al. that was attached as an Exhibit to Declaration "D" as made of record herein on June 9, 1995 (Nature, 347, 1990, pp. 382-386, which was received for publication by that journal on **July 27, 1990**). As described more fully in Declaration "D", this confirmation was accomplished by constructing clones in which various small fragments of the CFTR cDNA were placed upstream from a promoter-less reporter gene, and then monitoring for transcription/translation from the reporter gene to confer resistance in the presence of antibiotic. As shown in Figure 1 of Dr. Gregory's publication in Nature, the CFTR cDNA sequence fragment 905-950 confers substantial antibiotic resistance, and contains one of the two nucleotide subsequences (908-936) that lines up well with the E. coli consensus promoter sequence.

12. Applicants herein have thus disclosed multiple strategies for stable propagation of CFTR-encoding DNA in E. coli. Accordingly, Applicants fully believe that they are entitled to one or more patents for their important inventions, as represented by the claims that are now pending herein.

Part (B) — Introduction to the Non-Enabling Disclosures

13. Recent review of my notes taken at two conferences on cystic fibrosis research that were held during the period of April-June 1990 has indicated the making at those conferences of certain remarks by Dr. Lap-Chee Tsui, a co-inventor/co-author of many of the documents found in the Exhibit Book submitted herein on June 9, 1995, concerning his then-current attempts to construct a full-length CFTR-encoding cDNA. I believe that Dr. Tsui's remarks presented a very confused picture of his in-progress experiments, particularly when the remarks are viewed in the context of contemporaneous statements made at those conferences by others with whom Dr. Tsui had collaborated extensively, and who are also co-authors/ co-inventors of art references that are relevant herein.

14. The oral remarks of Dr. Tsui that are referred to herein were made at either:

(A) the conference "Identification of the CF Gene: Recent Progress and New Research Strategies", at Sestri Levante (Genoa), Italy held April 9-11, 1990, organized by the International School of Pediatric Sciences, Istituto G. Gaslini (Genoa) and sponsored by the Fondazione Internazionale Menarini (Milan), and the Cystic Fibrosis Foundation (USA), hereinafter the "April 1990 conference"; or

(B) the conference "The Gene-Nine Months Later", at Kingsmill Resort and Conference Center, Williamsburg, Virginia held June 10-13, 1990, sponsored by the Cystic Fibrosis Foundation (USA), hereinafter the "June 1990 conference".

15. I believe that no formal written record exists of the Williamsburg conference. With respect to the April 1990 conference, written record of all of the main presentations, and also written record of a few of the spontaneous "floor discussions" that occurred after certain individual presentations, were preserved in the book, "The Identification of the CF(Cystic Fibrosis) Gene—Recent Progress and New Research Strategies", Advances in Experimental Medicine and Biology, vol. 290, L-C. Tsui, ed., Plenum Press, New York, 1991.

16. Pursuant to Rule 1.56 and by way of the further Supplemental Information Disclosure Statement that is being submitted herewith, relevant sections of the above-mentioned monograph are provided to the Examiner, as are copies of relevant portions of my handwritten notes taken at both conferences. By this Declaration, I demonstrate that the above-mentioned oral remarks of Dr. Tsui of April and June 1990 were conflicting and unclear, and therefore did not disclose or enable any invention to those in the art-skilled audiences. Proof of this is provided, in part, by making reference to the British patent application No. 9020632.7 of Dr. Tsui et al. of September 21, 1990, wherein the numerous serious technical mistakes that still affected their "inventions", even when these "inventions" came to be described in such a detailed writing, are open to inspection. In fact, the text of this British patent application clearly shows that those authors were - even in September of 1990 - not in possession of an invention relating to CFTR-encoding cDNA stabilized for propagation in E. coli, and in fact were actively teaching away from it.

Part C — The British Patent Application filed in September of 1990

17. Document (G) of the Exhibit Book, as made of record herein on June 9, 1995, is Canadian PCT application No. PCT/CA91/00341 of Dr. Tsui et al. published on April 2, 1992 claiming priority to Great Britain national application 9020632.7, itself filed September 21, 1990. The filing of this British priority application (also provided in the Supplemental Information Disclosure Statement submitted herewith) coincided with publication - that very same day - of Dr. Tsui's work (and that of his co-inventors/co-authors) in the journal Cell (at volume 62, pp. 1227-1233 thereof, see Document (I) in the Exhibit Book). As such, the British national application, as it is also reflected in the text of the Canadian PCT application which is derived therefrom, provides a most up-to-date window to what those authors/inventors knew in September of 1990 and what they did not. This document also provides insight into their earlier oral remarks made at the April and June 1990 conferences.

18. In this regard, the Examiner's attention is respectfully directed to pages 17-18 and also Figure 3 of Document (G) in the Exhibit Book entitled "Stable Propagation of Modified Full Length Cystic Fibrosis Transmembrane Conductance Regulator Protein cDNA in Heterologous Systems". At page 17, Dr. Tsui et al. describe full length CFTR-encoding cDNA constructs that they have prepared. The constructs contain 3 point mutations of interest: T to C at nucleotide 930, A to G at 933, and T to C at 936.

The T to C mutation at nucleotide 936

19. **Twice** on page 17 of Document G (at lines 17 and 19 thereof), the T to C mutation at position 936 was described as being introduced into the cDNA by **"an error"** . The 936 mutation was also denominated an **"unanticipated alteration"** (at line 16) which was positioned **outside of a conserved region** of the prokaryotic RNA polymerase consensus sequence (see lines 19-21). The stated uselessness of the T to C mutation at position 936 was further underscored therein when Dr. Tsui referred to it as **"undesired"** (line 22). Clearly (see line 21), the only reason this **"undesired-unanticipated-error"** was allowed to remain in the construct was because it was expected to be harmless, i.e. it did not alter the amino acid encoded by the mutated codon. Page 17 clearly teaches that the invention described in Document (G) — alleged stabilization of cDNA for propagation in bacteria— must result from the point mutations at positions 930 and 933, the only mutations that those inventors claim to have "intended".

20. In fact, mutation of position 936 is **a key mutation** that stabilizes CFTR-encoding DNA for propagation in E. coli. That such a mutation would be very useful was developed by Dr. Gregory and his co-inventors at Genzyme prior to **July 27, 1990**, the date that their publication evidencing this important discovery (Nature, 347, pp 382-386) was received by that journal for publication. The Nature article is attached to Dr. Gregory's Declaration "D" submitted herein on June 9, 1995, and page 1 at column 1 thereof, and Figure 1 show why the 936 mutation is so effective in stabilizing CFTR-encoding DNA for propagation in E. coli.

21. As aforementioned, Dr. Gregory and his co-inventors had determined that nucleotides 908-936 of the CFTR-encoding cDNA show strong homology with the nucleotide sequence recognized to represent the consensus for an E. coli RNA polymerase promoter. The consensus sequence is:

5′ TTGACA ---- gap of 17+/- 1 base pairs ---- TATAAT 3′ ,

as depicted in the Nature article, and is also depicted at page 9 of the Reznikoff and McClure article that is cited in Example 5 of our patent application (see also Example 6 of our 07/488,307 parent application filed March 5, 1990). The Reznikoff and McClure article is being provided to the Examiner herewith via a Supplemental Information Disclosure Statement, and a copy of page 9 thereof is attached directly to this Declaration for the Examiner's convenience.

Inspection of the CFTR cDNA sequence shows that nucleotide position T936 corresponds to a most highly conserved position in the consensus (the 3′ end "T" in TATAAT, see also the Nature article of Dr. Gregory et al.), so that mutation thereof is expected to decrease substantially the likelihood that this subsequence of the CFTR-encoding cDNA would be recognized as a bacterial RNA polymerase promoter.

22. As is clearly apparent, however, from Figure 3 of Dr. Tsui's Canadian PCT application (Document G in the Exhibit Book), that application explicitly and unavoidably teaches away from the great utility of the 936 mutation because—in that application—nucleotide position 936 falls outside the misidentifed 5′ conserved region of the misidentified "promoter" depicted therein. It follows directly that the Canadian PCT application must teach- as indeed it conceeds on its face - that mutation at position 936 has no useful purpose. Simply stated, Tsui. et al. cannot find the promoter.

23. As explained below, the position 930 and 933 point mutations made by Dr. Tsui et al., as described in their British patent application of September 1990, are not mutations that one skilled in our art would recognize as being useful to stabilize a CFTR-encoding DNA for propagation in E. coli. In fact, these mutations were only made as a result of several erroneous assumptions (see below) made by Dr. Tsui and his co-inventors/co-authors:

(A) The evidence demonstrates that Dr. Tsui believed that recombination at direct repeats in exon 6 was responsible for the cloning difficulties, not the presence therein of a cryptic bacterial RNA polymerase promoter.

(B) Even after learning from Genzyme researchers (see below in reference to the April 1990 conference) that a cryptic promoter was the likely cause of the cloning difficulties he had encountered, Dr. Tsui could not locate the promoter. This is clearly evident in Figure 3 of the Canadian PCT application (Exhibit Book, Document G) wherein Dr. Tsui's "promoter" is depicted as beginning at CFTR cDNA nucleotide 929 and running to nucleotide **958**. This is clearly incorrrect, as shown by Dr. Gregory's experiments in the above-mentioned Nature publication wherein gene expression (Figure 1 thereof) was achieved using, as presumptive promoter, a nucleotide known as pKK-oligo 3 which is the nucleotide sequence 905- **950** of CFTR cDNA, and includes the 908-936 promoter-capable sequence. Having misaligned the promoter in the CFTR cDNA, Dr. Tsui and his co-inventors/co-authors were unavoidably destined to teach selection of the wrong nucleotides for proposed alteration in the cDNA. In this regard, the Examiner's attention is respectfully directed to Page 39 of Document (G), the Claims, wherein **the T936C mutation is neither mentioned nor claimed**.

The T to C mutation at nucleotide 930

24. In comparison with Dr. Tsui's disclosures concerning the "making" of a mutation at CFTR nucleotide position 936, Dr. Tsui's disclosures pertaining to mutation at nucleotide position 930 reflect different— but equally erroneous — assumptions. Nucleotides 931-936 (GAAAAT) of the CFTR cDNA correspond (see Figure 1 of the Gregory et al. Nature article) to the 3' end of the E. coli consensus sequence (TATAAT). Simply stated, **nucleotide position 930 is, in fact, outside the 3' conserved region of the true promoter**. Accordingly, the T to C mutation at position 930 of the CFTR-encoding cDNA is not expected to have any effect on promoter recognition and DNA stability in E. coli.

25. I believe also that Dr. Tsui and his co-inventors/co-authors have never performed any experiments to create a DNA construct containing only a 930 mutation, and I can think of no basis for expecting that such a construct would stabilize the cDNA for propagation in E. coli. Instead, those skilled in our art would recognize (based on the teachings of Dr. Gregory's paper in Nature, and which were not publicly available prior to that paper's September 27, 1990 publication date) that any stabilizing effect contributed by Dr. Tsui's triple-mutant construct would be provided, in fact, by the **"unanticipated" "undesired" "error"**, i.e. the 936 mutation, that that construct accidentally contained, but which it was taught (page 17 of Document G) was not part of that invention.

26. It is clear also that Dr. Tsui and his colleages would never have suggested the making of a point mutation at position 930 but for the fact that they had misaligned the promoter, thereby placing nucleotide 930 inside the conserved 5' end of their "promoter", when in fact it is just outside the conserved 3' end of the true promoter [in Dr. Tsui's incorrect scheme as presented in Figure 3 of Document (G), position 930 (a "T") improperly corresponds to the second T in the 5' end sequence TTGACA (Reznikoff and McClure, page 9) of the consensus promoter].

The A to G mutation at nucleotide 933

27. A similar analysis may be performed with respect to the A to G point mutation at nucleotide position 933 that was disclosed in Dr. Tsui's Canadian PCT application (and its British priority document). Position 933 in the CFTR cDNA corresponds in fact, based upon the correct alignment as provided in the Gregory et al. Nature publication, to the second "T" in the 3' conserved end of the consensus, TATAAT.

However, and referring also to page 9 of the Reznikoff and McClure reference, this "T" is the least conserved nucleotide in the consensus (i.e. mutation at this position is predicted to have the least effect on whether the resultant sequence is, or is not, recognized as a promoter). Additionally the consensus that one seeks to avoid at this position is **"T"** whereas the corresponding nucleotide in wild type human CFTR cDNA is already **"A", not "T"**. Since the wild type CFTR 933 nucleotide "A" does not correspond to the recognized consensus nucleotide "T", and this nucleotide position is the least conserved one in the 3' end of the consensus to begin with, it is my view that no one skilled in our art would seek to change wild type nucleotide 933 with an expectation of success (i.e improved propagation in E. coli).

28. There is further reason that a report of mutation at position 933 in the CFTR cDNA (see pages 17-18 of Document G) would not have been recognized by those skilled in our art as having any purpose. According to the misaligned promoter scheme that Dr. Tsui presented in Figure 3 of Document (G), the consensus nucleotide (see also page 9 of the Reznikoff and McClure reference) that CFTR nucleotide 933 is supposed to correspond to — and that one therefore seeks to avoid — is the **"C"** in 5' TTGACA ---- . However, since this nucleotide is already an **"A"** in wild type CFTR cDNA, it is entirely unclear what is supposed to be accomplished in that scheme by mutating it to **"G"**. Accordingly, disclosure of the making of this mutation provides no useful information to the art-skilled scientist.

29. Additionally, there is no evidence of which I am aware that Dr.Tsui (or any of his co-authors/co-inventors) has ever performed an experiment in which **the only** point mutation made in a CFTR- encoding cDNA was made at position 933, or at position 930, or a combination of both. As discussed above, those skilled in the art would predict that such mutations would be of no consequence, and that any such cDNA containing only these mutations would remain toxic to host E. coli cells.

30. In fact, the only experiments that have ever been reported that actually identify a particular nucleotide position in the CFTR cDNA - mutation of which permits stable propagation of the cDNA in a bacterium - are the experiments of Genzyme inventors Gregory et al. that involve the 936 T to C mutation (see Example 7, page 17, of Applicants' pending patent application, and also Nature, 347, 1990, pp. 382-386, as cited above).

31. Accordingly, those skilled in the art would not recognize the disclosure provided by Dr. Tsui et al. in Canadian PCT application PCT/CA91/ 00341 (or in its abandoned British priority document) as providing any recognizeable teaching as to how CFTR cDNA can be stabilized for propagation in E. coli using point mutations. This is particularly so since the use of the 936 mutation, which represents in fact a very important solution to the problem of stable propagation in bacteria, is so thoroughly discredited in that application **as soon as it is "mentioned"**.

> Tsui et al. may have made the mutations to avoid homology between two direct repeats in the CFTR cDNA— but this was not the cause of the cloning difficulties.

32. It is also my judgment that the nucleotide changes made by Dr. Tsui et al. were never made with the intent to inactivate a cryptic promoter, but instead were made with the intent of preventing homology between two direct repeats (nucleotides 923-935, and 981-993, Document G at page 12 thereof) recombination at which, Dr. Tsui believed (see below), was the source of the cloning difficulties that prevented propagation of full length CFTR-encoding DNA in bacteria. The present Applicants had, early on, ruled out this incorrect theory as evidenced, for example, by Example 1 of our 07/488,307 patent application filed on March 5, 1990 wherein we stated that:

> Initial attempts to reconstruct the entire CFTR protein coding sequence in high copy number plasmids similar to those reported by Riordan et al., produced only molecules with internal rearrangements and deletions of coding sequence. Such rearrangements can result, for example, from recombination catalyzed by host cell proteins, and they occur primarily between regions of complete or partial nucleotide sequence identity within a DNA molecule, such as are present at direct or inverted repeat sequences. Although such deletions could be attributed to instability of the CFTR cDNA in E. coli, computer analysis of the CFTR sequence did not reveal an unusual number of direct and inverted sequence repeats within the published CFTR sequence, **thereby indicating that the CFTR cDNA should not be inherently unstable in E. coli**. (emphasis added)

33. It follows directly that if the detailed September 1990 patent application of Dr. Tsui et al. is incorrect on key points, and confusing to those skilled in our art, then the oral remarks made at the April 1990 and June 1990 conferences by Dr. Tsui, or others having commonality of prior inventorship/authorship with him, could hardly be other than a less complete version of the same confusion. Those oral remarks are further discussed in the remainder of this Declaration.

Part (D) — Dr. Tsui's Oral Presentations of April and June 1990

34. The Examiner's attention is therefore respectfully directed to the following remarks of Dr. Lap-Chee Tsui, and of co-inventor/co-author Dr. Francis S. Collins, made at the above-mentioned conferences in April and in June of 1990 (whether reflected in the text of pre-prepared remarks or as taken from the floor discussions), as later published, or as remembered by me presently, or as reflected in my notes taken during those conferences. **(All reference numbers below refer to references provided in the Supplemental Information Disclosure Statement submitted herewith).**

(A) At page 44 of Reference **3** (refering to the April 1990 meeting), it is recorded that Dr. Tsui had no information that a cryptic bacterial RNA polymerase promoter was present in his clones. "We don't know whether it is. The cloning vectors we use are supposedly not expressing the protein, so I don't know whether there are any other cryptic promoters making a protein in these constructs."

(B) At page 14 of Reference **1**, under the heading "Protein Analysis" within his April 1990 presentation, Dr. Tsui stated "In this construct, we have altered nucleotide sequences within exon 6 to avoid frequent rearrangement **associated with** a 15 bp **direct repeat in this region**." (emphasis added)

(C) At page 44 of reference **3** (again referring to the April 1990 conference) it is recorded that Dr. Collins stated that a mutation in exon 8 also stabilized CFTR cDNA, but this appeared clearly to involve a construct in which the reading frame was frameshifted, so that no CFTR polypeptide could be encoded therefrom.

(D) At page 44 of Reference **3** it is recorded that Dr. Collins thought exon 6 of CFTR-encoding DNA contained a prokaryotic promoter. However, I distinctly remember that Dr. Collins also stated (and this is reflected in my notes) that "low copy number" - a procedure that might be used to avoid the very consequences of such a promoter— did not work. [The self-contradictory nature of his views is further apparent from — **and supported by** — Document (H) in the Exhibit book of June 9, 1995 which is an abstract that Dr. Collins and others were submitting at about the same time. The text of this abstract confirms that it was Dr. Collins' conclusion that low copy number methodology would not work.]

Dr. Collin's position would therefore be recognized by those skilled in the art as unclear and apparently self-contradictory. Accordingly, and in order to correct this misunderstanding during the floor discussions at the April 1990 conference, Dr. Kathy Klinger of our company announced that the presence of a bacterial consensus (promoter) sequence in the CFTR cDNA was responsible for the cloning difficulties, and that low copy number did work . In fact, we had already used low copy number to perform the experiment that Dr. Collins and Dr. Tsui and all of their colleages could not do (see our priority U.S. patent application 07/488,307 filed March 5, 1990), that is, to assemble full length CFTR-encoding cDNA and then propagate it in E. coli.

(E) As aforementioned and in contrast, Dr. Tsui clearly had believed that the properties of a pair of 15 bp direct repeats within exon 6 of the CFTR-encoding DNA were responsible for the cloning difficulties in bacteria (see Reference **1**, referring to the April 1990 conference, at page 14 thereof, under the heading "Protein Analysis" and also pages 17-18 thereof.

(F) Reference **1**, also at page 14 thereof, under the heading "Protein Analysis" also records mention by Dr. Tsui that he had reconstructed a full length cDNA by making nucleotide alterations in the exon 6 region. However, the exact nature of the construct was not recorded, and the construct was presented in the context of "solving" Dr. Tsui's "direct repeat problem" — which problem , in fact, was not responsible for the cloning difficulties that Genzyme researchers had, by more than one method, already resolved.

(G) Reference **4** (my notes of the April 1990 conference) appears on review thereof to indicate that Dr. Tsui asserted that he had a prepared a "full length" cDNA, and also that the resulting clones were more stable. The region of the cDNA that was changed apparently encoded the amino acids "Ileu-Glu-Asn", but there are two such subsequences in the CFTR protein (the two direct repeats from within exon 6), and I believe that there was no disclosure of which particular encoding nucleotides therein were being changed. In fact, Dr. Tsui maintained even much later, see paragraph (I)(i) below, that mutating **either** of the direct repeats was effective, which is not consistent with the now-established explanation of the cloning difficulties — the presence of the cryptic promoter. Accordingly, I believe that the art-skilled audience would have been unable to determine which alleged theory of clone instability was being addressed. Additionally, my notes reflect that the CFTR polypeptide apparently encoded thereby would have had (owing to how it was constructed) at least one amino acid mutation (position 2, directly after the intiator methionine), and there was no showing that any protein having functional chloride channel activity had been produced.

(H) Reference **5** provides my notes taken during Dr. Tsui's talk at the June 1990 conference on cystic fibrosis in Williamsburg, Virginia. My notes reflect mention by Dr. Tsui of a "full length" construct that encoded for a CFTR polypeptide having at least one mutated amino acid (position 2, Gln to Glu). According to my notes, he was still trying to do the "protein experiments", that is, identify the protein encoded thereby.

(I) Also during his June 1990 presentation at Williamsburg, Dr. Tsui made mention of particular point mutations that he had made into CFTR cDNA. In doing so he presented a very confused picture owing to the following facts and related circumstances.

(i) As demonstrated above, Dr. Tsui had always maintained that inactivation of the direct repeats in exon 6 was essential to the practice of his invention. This is clearly recorded in Exhibit Book Document (G), which reflects Dr. Tsui's thinking as of September 1990. At page 12, paragraphs 2 and 3 thereof, **his adherence to this theory is indicated by the teaching** that mutation of **either** of the direct repeats (nucleotides 924 to 936, **or** 982 to 994), but without changing the encoded amino acids, results in stable bacterial propagation. The repeats both involve Met-Ileu-Glu-Asn (see Riordan et al. 1989), although the first Asn residue (both repeats end within their Asn codons) is encoded from "AAT" whereas the second Asn is encoded from "AAC". In fact, mutation at the second of the repeats is expected to be inoperative since it would fail to inactivate the cryptic promoter.

(ii) As provided in my notes of the June 1990 conference (Reference **5**, attached), Dr.Tsui mentioned nucleotide substitutions that appear to be associated with one of his stated full length constructs. The mutations are underlined in my notes. However, the making of the (Asn) AAT to AAC mutation was inconsistent with the direct repeat theory that Dr. Tsui had presented earlier, because in making this mutation both Asn residues (one in each repeat) came to be encoded by AAC, **so that even more direct repeat would have been created making the problem worse, not better.** Accordingly, it was impossible to understand what the totality of Dr. Tsui's remarks were supposed to mean — given all that was presented. Dr. Tsui's Canadian PCT application (Document G, see also its September 1990 British priority document) is some 40 pages long, contains 20 figures, and is substantially directed to this very subject; however, it does nothing to clarify these confusions. Rather it introduces further confusion indicating that those authors simply did not know, and could not therefore explain to others, what they had.

35. Accordingly it is apparent that the information orally disclosed at the April and June 1990 conferences by Dr. Tsui, and co-authors/co-inventors having commonality with him, was confusing- to say the least- when taken as a whole, and did not describe any inventions to the skilled audiences present at those meetings. Since the data presented was, when taken as a whole, inconsistent with inactivating a direct repeat, and was inconsistent with inactivating a cryptic promoter, one could simply not determine what to make of their ideas.

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36. All of the inventions disclosed in our present patent application, and in all of the parent applications thereof, were conceived of and made in the United States.

37. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of United States Code, and that the making of any such willful false statements may jeopardize the validity of this application, of related applications, and of any patent issued thereon.

Date: 8/14/95

Declarant: 
Alan E. Smith, Ph.D.

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is shown in Figure 1–3. The most highly conserved bases in the two regions of the promoters are shown by the size of the individual letters. It is remarkable that the consensus sequence has changed only slightly with additional promoter sequences added to previous compilations (Rosenberg and Court 1979; Siebenlist et al. 1980). Several important conclusions derived from the current compilation can be stated in the form of rules for RNA polymerase recognition of *E. coli* promoters. It is clear that the TTG in the –35 region and the TA---T in the –10 region are the most highly conserved base pairs within the promoter. Other bases of varying significance in a statistical sense occur on either side or within these sequences. One rule that was deduced on the basis of this compilation is that the consensus sequence is best. The rule is supported by the location and identity of more than 100 promoter mutations. The sequences of promoter mutations show that base pair alterations that decrease homology to the consensus sequence are down-promoter mutations and that base pair alterations that increase homology to the consensus sequence are up-promoter mutations. The only exceptions to the rule are a handful of nonconsensus-to-nonconsensus base pair alterations that suggest the possibility of a hierarchy of recognition at each position within the promoter. Another rule that can be applied to all the sequenced *E. coli* promoters is that each known promoter shows a match of at least two out of three base pairs at the three most highly conserved sites within the –10 region. The T residue at –7 has frequently been referred to as "invariant." That seems not to be the case. Indeed, the A residue at –11 appears to be nearly as invariant as the –7 T. There is considerably more diversity in sequence in the –35 region, and the less stringent rule that applies is that every known *E. coli* promoter has a match of at least one out of three to the highly conserved TTG sequence in that region.

In addition to the homologies found in –35 and –10, another feature of the promoter has been shown to be important in function—namely, the spacing between the –35 and –10. The distance as conventionally expressed is ordinarily 17± 1 base pairs. Again, the evidence from mutations is that the

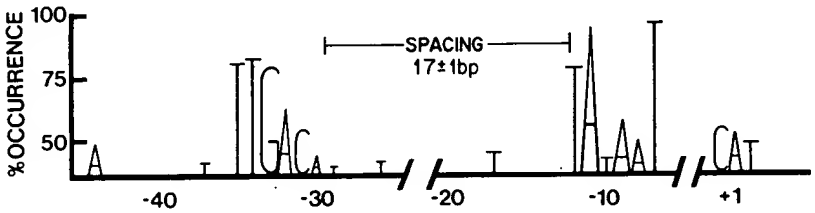

FIGURE 1–3 The consensus sequence for *E. coli* promoters. Each base on the top strand is displayed in height corresponding to its percent occurrence in the 112 promoters compiled by Hawley and McClure (1983). The baseline corresponds to a level two standard deviations (Poisson statistics) above that expected for random occurrence of bases at each position.